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The Gametophyte of *Diphasiastrum sitchense*

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ABSTRACT.—The spores of *Diphasiastrum sitchense* germinate in the dark on a nutrient medium containing inorganic nutrients and glucose. Dark-grown prothalli develop into white, carrot-shaped gametophytes with a tapering base, constricted neck, and gametangial cap. The antheridia are large and sunken, and the archegonia have long necks with numerous neck canal cells. The tapering base has a zone of radially elongated cells that is comparable to the inner mycorrhizal zone of *Diphasiastrum* gametophytes from nature. Although possessing few derived sporophytic characters, *D. sitchense* has a typical carrot-shaped, *Diphasiastrum* gametophyte.

The sporophyte of *Diphasiastrum sitchense* (Rupr.) Holub is considered to be the most basal member of this genus in North America (Lloyd, 1901; Marie-Victorin, 1925; Wilce, 1965; Tryon and Moran, 1997). The main reason for this conclusion is the type of leaf and their arrangement on the stem. The leaves of *D. sitchense* are isomorphic and spirally arranged on terete branchlets compared to the di- or trimorphic leaves and decussate arrangements on flattened branchlets of the remaining North American members of the genus (Wilce, 1965). In addition, an analysis of many characters has shown that *D. sitchense* has next to the fewest number of derived characters for the genus worldwide (Wilce, 1965).

The known gametophytes of *Diphasiastrum* are from species having sporophytes with many derived characters. The gametophytes of these species are subterranean, mycorrhizal, and carrot-shaped (Bruchmann, 1908; Bruce, 1979; Whittier, 1981). Because gametophytes from the basal members of the genus are unknown, it would be of interest to determine if the gametophyte morphology of *D. sitchense* is different from those of the species with derived sporophytic characters.

This study was carried out to determine the type of gametophyte in *D. sitchense* using the techniques of axenic culture. It has been over 150 years since this taxon was recognized, however, no gametophytes have been collected from natural areas. For this reason, growing these gametophytes in culture provided an opportunity to determine the structure of this gametophyte.

MATERIALS AND METHODS

Spores of *Diphasiastrum sitchense* were obtained from strobili collected during September in King County, Washington and Lane County, Oregon. Vouchers of the King Co. plants are on deposit at VDB and those of the Lane Co. plants (*D. H. Wagner #m0732*) are on deposit at OSC.

The spores were surface sterilized with 20% Clorox (1.1% sodium hypochlorite), following the techniques of Whittier (1973) and were sown on

15 ml of nutrient medium in 20 × 125 mm culture tubes with screw caps that were tightened to reduce moisture loss. The sown spores were maintained in darkness or under a 14 hour photoperiod (50 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) from Gro-lux fluorescent lamps at 21 ± 1°C.

The nutrient medium contained 100 mg NH_4Cl, 50 mg $MgSO_4 \cdot 7H_2O$, 20 mg $CaCl_2$, and 50 mg K_2HPO_4 as a final concentration per liter. A liter of the medium was completed with 0.25 ml of a minor element solution (Whittier and Steeves, 1960), 4 ml of an FeEDTA solution (Sheat *et al.*, 1959), and 5 g of glucose. The medium was solidified with 1% agar and was at pH 5.2 after autoclaving.

The gametophytes were fixed with Randolph's Modified Navashin Fluid (CRAF; Johansen, 1940). After fixation, the gametophytes were embedded in paraffin and sectioned by conventional techniques (Johansen, 1940). The sections were stained with Heidenhain's hematoxylin, safranin O, and fast green.

Results

After 5 months in the dark about 0.5% of the spores germinated (Fig. 1). Germination never exceeded 1% with more time in the dark. No spores had germinated in illuminated cultures after 11 months.

Young multicellular gametophytes were found after 8 months. These small, globular gametophytes usually had spore coats attached (Fig. 2). At nine months, larger globular gametophytes were transferred to fresh nutrient medium for further growth. Mature gametophytes were obtained 9 months after this transfer. The oldest gametophytes studied were collected 2 years after sowing the spores.

Mature gametophytes were white and carrot-shaped (Figs. 3, 4, 5) and the largest found were about 8 mm long. The upper and basal regions of the gametophytes were separated by constricted necks. This constriction (Figs. 3, 4, 5, 6) is the site of the meristematic region (ring meristem) in gametophytes of *Diphasiastrum*. The more or less conical basal region was covered with numerous rhizoids. The upper region, the gametangial cap, was the site for antheridia and archegonia. The gametangial caps on young gametophytes produced antheridia first, followed by the formation of archegonia. On mature gametophytes, antheridia were in the middle of the gametangial cap surrounded by archegonia.

The archegonia were prominent when present. They had long necks usually with 9–12 neck canal cells (Figs. 6, 7). Neck length, from base of egg cell to tip of neck, averaged 274 μm. The antheridia were large and sunken (Fig. 8). The elongated sperm masses averaged 233 μm long and 118 μm wide. Large numbers of male gametes were formed by each antheridium.

Besides being the site for rhizoid formation, the tapering basal regions of *Diphasiastrum* gametophytes from nature house a mycorrhizal fungus. In axenic culture the gametophytes grow without the mycorrhizal fungus if sugar is available in the nutrient medium. However, the basal regions of the gametophytes from axenic culture did develop some anatomical features found in

gametophytes of other species from nature. Sections show elongated cells close to the basal surface of these gametophytes (Fig. 9). These cells are in essentially the same position as the elongated cells of the inner mycorrhizal region of gametophytes of other *Diphasiastrum* species. Thus, aspects of a mycorrhizal region differentiated in these gametophytes in the absence of a fungus.

Discussion

The gametophytes described for *Diphasiastrum* are Type II (Bruchmann, 1898). The Type II gametophytes are carrot-shaped with an upper area, the gametangial cap, separated from the tapered basal region by a constricted neck with a ring meristem. The gametangia of these gametophytes are larger than those found in most of the other gametophyte types described for *Lycopodium sensu lato* (Bruchmann, 1898). The antheridia are massive and sunken into the gametangial cap (Bruce, 1979). The long-necked archegonia have the largest number of neck canal cells reported for any of Bruchmann's gametophyte types.

The gametophyte of *D. sitchense* fits the description for the gametophytes (Type II) of this genus (Bruchmann, 1908; Bruce, 1979; Whittier, 1981; Whittier and Britton 1995). There is nothing unusual about the gametophyte of *D. sitchense*. It is carrot-shaped with all the described regions present. The antheridia are large, sunken structures in the gametangial cap and are similar in size to those described for *D. digitatum* (A. Braun) Holub and *D.* X*habereri* (House) Holub from axenic culture (Whittier, 1981; Whittier and Britton, 1995). The archegonia have long necks with large numbers of neck canal cells and they are similar in length to the archegonia of *D. digitatum* from soil and axenic culture (Bruce, 1979; Whittier, 1981).

The basal region of *Diphasiastrum* gametophytes from soil have a distinctive three layered mycorrhizal region (Bruce, 1979; Whittier, 1981). The development of a three layered mycorrhizal region did not occur in the gametophytes lacking an endophytic fungus. However, elongated cells form in the basal region of these gametophytes and they are in the correct position for the elongated cells found in gametophytes of *D. digitatum* (Bruce, 1979) and *D. complanatum* (L.) Holub (Bruchmann, 1898) from soil. Also, these elongated cells are in the same position as elongated cells in gametophytes of *D. digitatum* from axenic culture (Whittier, 1981). The tissues of the basal region of *D. sitchense* are very similar to those in other gametophytes of the genus.

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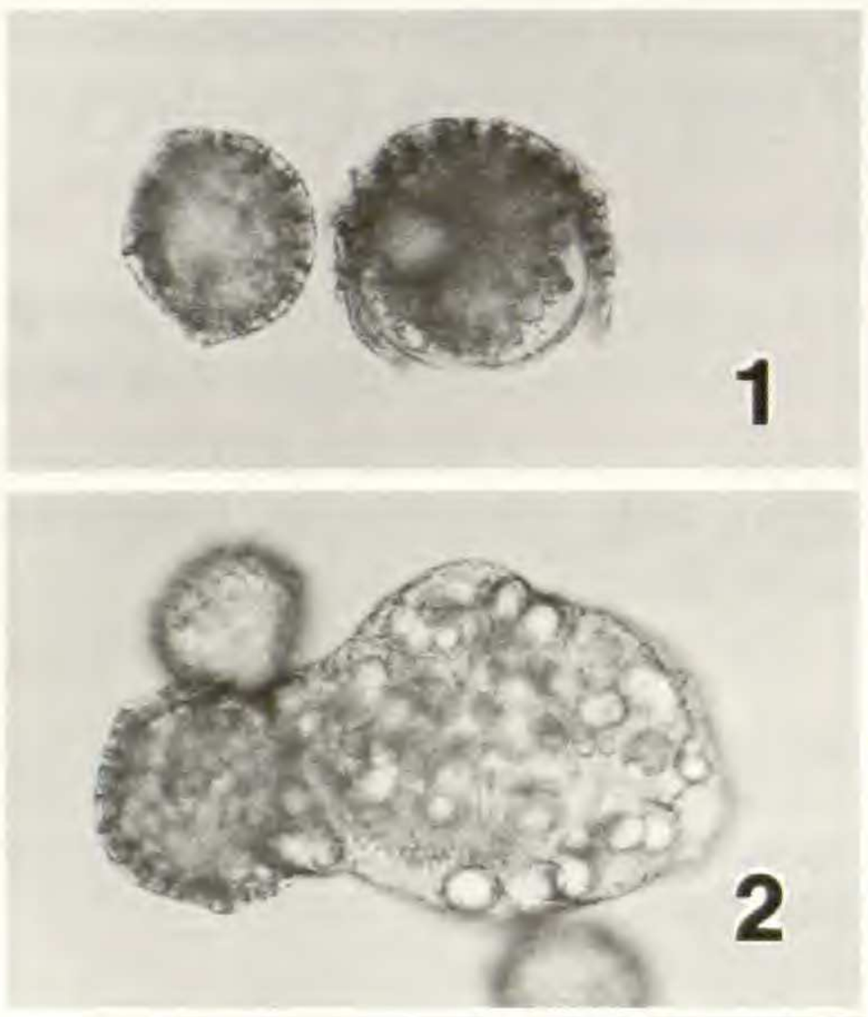
Figs. 1–9. Gametophytes of *Diphasiastrum sitchense*. Fig. 1. Germinating spore, 320×. Fig. 2. Young globular gametophyte, 255×. Figs. 3–5. Mature carrot-shaped gametophytes with gametangial caps, constricted necks (white arrowheads), and conical bases bearing rhizoids. Fig. 3. Gametophyte with small gametangial cap bearing mainly antheridia. Arrow indicates two archegonial necks, 7×. Fig. 4. Gametophyte with archegonia. 6×. Fig. 5. Gametophyte with archegonia, 10×. Fig. 6. Constricted neck of gametophyte with young short-necked archegonia (arrowheads) at lower edge of gametangial cap and mature long-necked archegonia (arrows) on gametangial cap, 16×. Fig. 7. Longitudinal section of archegonium, 130×. Fig. 8. Longitudinal section of antheridia, 130×. Fig. 9. Longitudinal section of conical base with elongated cells (arrows), 130×.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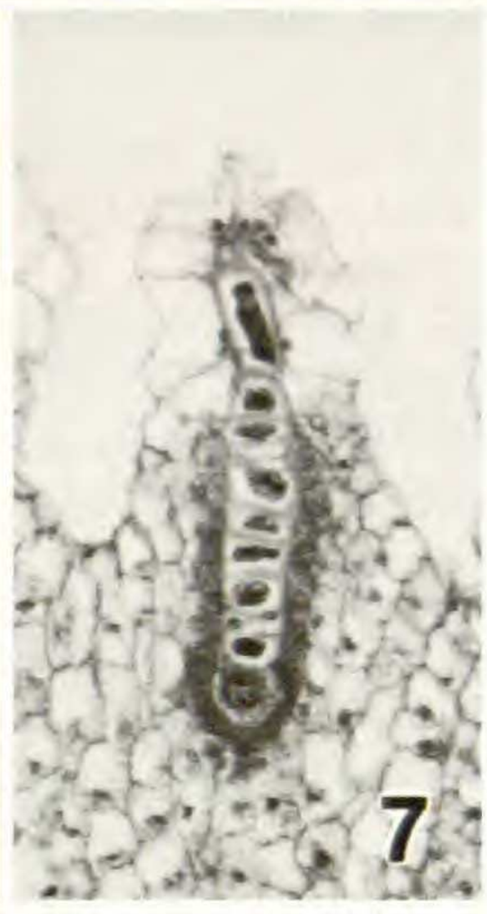
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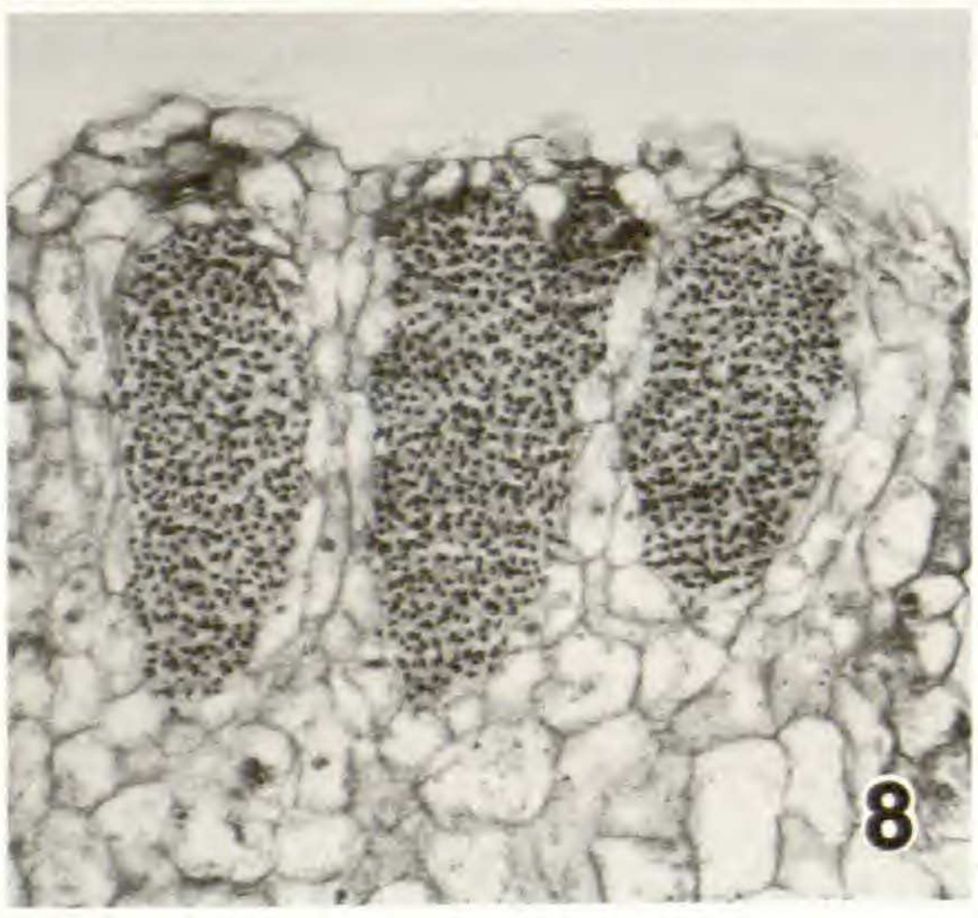
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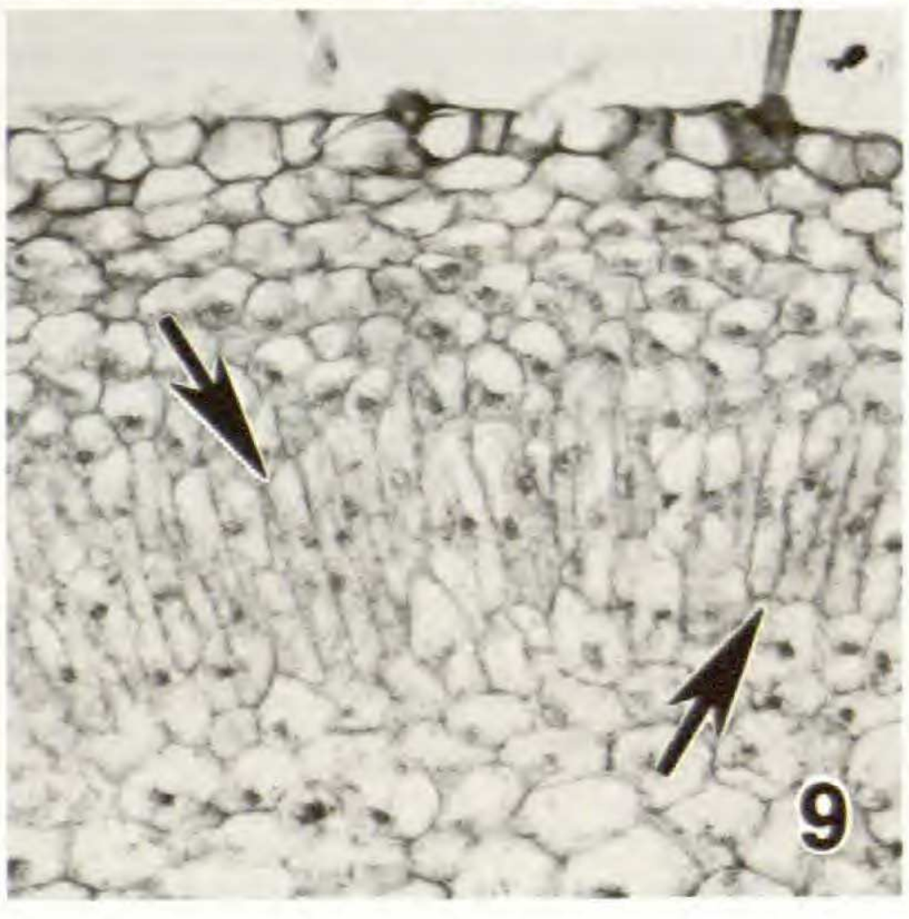
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The sporophytes of *D. sitchense* and *D. veitchii* (Christ) Holub, an Asian species, are different from other species of *Diphasiastrum*. They have almost all basal characteristics for the genus (Wilce, 1965). However, the gametophyte of *D. sitchense* is normal and typical for the genus.

Bruce (1979) had raised the possibility that gametophytes of *D. sitchense* and *D. veitchii* might be informative in bridging the structural differences between Type I and Type II gametophytes. This is not the case with the gametophyte of *D. sitchense*. Gametophytes of other species will have to be examined to determine if an intermediate condition can be found.

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